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TECHNICAL MANUSCRIPT 224

ETHYLENE PRODUCTION  
FROM LINOLENIC ACID

Frederick B. Abeles

MAY 1965

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U.S. ARMY BIOLOGICAL LABORATORIES  
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TECHNICAL MANUSCRIPT 224

ETHYLENE PRODUCTION FROM LINOLENIC ACID

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Crops Division  
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1A014501A91A

May 1965

ABSTRACT

The possibility of linolenic acid serving as the precursor of ethylene in vivo was investigated. A comparison of the amount of linolenic acid present in plant tissues with the rates of ethylene production indicated that linolenic acid does not serve as the source of ethylene in plants.

## ETHYLENE PRODUCTION FROM LINOLENIC ACID

Lieberman and Mapson<sup>1</sup> have shown that linolenic acid may serve as a precursor of ethylene. The possibility that the ethylene that is evolved from plants arises from linolenic acid was investigated by determining the relationship between ethylene evolution and linolenic acid levels from a number of plants.

Ethylene production from linolenic acid was measured in a manner similar to one described earlier.<sup>2</sup> The fatty acid peroxides were measured according to the methods of Dahle et al.<sup>3</sup> Extraction and measurement of fatty acids from plants followed the method of Huston and Albro.<sup>4</sup> Ethylene evolution from intact plants was measured by placing material into three-liter containers and withdrawing samples of gas after 20 hours. Measurement of ethylene by gas chromatography has also been described earlier.<sup>5</sup> Etiolated beans (Phaseolus vulgaris L. var. red kidney) and peas (Pisum sativum L. var. alaska) were grown in the dark for a week at 25 C and 80% relative humidity. Apples (Malus pumila Mill. var. eastern delicious) were obtained from local markets.

Figure 1 presents the results of a representative experiment showing the production of ethylene from peroxidized methyl linolenate. Other hydrocarbon gases such as ethane and propane, and a number of unidentified peaks were also produced. The presence or absence of a methyl group on the fatty acid has no effect on ethylene production. Under the conditions shown in Figure 1, the amount of ethylene evolved after 24 hours represents a 0.2% yield on a mole of ethylene per mole of peroxidized emolinate basis. The effect of ascorbate as a catalyst could be duplicated by 0.1 mg of a crude pea protein fraction described earlier<sup>2</sup> or similar amounts of lipoxidase. In these cases the activity of the protein was destroyed by heating. Addition of 1  $\mu$ mole of KCN and 0.25  $\mu$ mole of ethylenediamine-tetraacetic acid inhibited gas production. A pH optimum between 7 and 8 was obtained with phosphate buffer; other buffers, such as pyrophosphate, tris(hydroxymethyl)aminomethane, and glycylglycine lowered the rate of gas production.

The possibility that ethylene production from peroxidized linolenate was mediated by means of a free radical mechanism was tested by the addition of 10  $\mu$ moles of catechol or  $I_2$ . Both of these compounds are known to quench free radical reactions by preferentially trapping the unpaired electrons. Both catechol and  $I_2$  inhibited ethylene production from pea protein and ascorbate catalyzed reactions. In the case of the pea protein reaction inhibition was about 70% and for the ascorbate reaction inhibition was about 85%.

Other fatty acids were examined for their ability to serve as a substrate for this reaction. Stearic, oleic, linoleic, and arachidonic were found to be inactive. However, a C 22 fatty acid from herring oil, clupanodonic acid, was found to be active. The similarity between this compound and linolenic acid is the presence of a terminal  $\text{CH}_3\text{-CH}_2\text{-CH=}$  group at the methyl carbon end of the chain. On the premise that this particular end group accounts for the activity of linolenic acid, other compounds having the same structure were examined. 2-Pentenoic acid, 3-hexenoic acid, and  $\beta$ -heptene were also active as a source of ethylene. Under conditions identical to those in Figure 1, ethylene production from these compounds was in the order of one nl (nanoliter) ethylene after 30 minutes.

Levels of linolenic acid and rates of ethylene production from apples were determined to explore the suggestion of Lieberman and Mapson<sup>1</sup> that ethylene production from apples may arise from linolenic acid. Market apples were peeled, cored, and sliced into quarter sections. Two slices were stored in a freezer and the other two placed in a container and the ethylene evolution measured after 20 hours. A representative chromatogram of the fatty acids extracted from apples is shown in Figure 2. The data from two experiments in Table 1 indicate that the linolenic acid content of apples remains approximately constant during this 24-hour period. The gas production during this time is equivalent to about 20% of the linolenic acid content. If linolenic acid is the source of ethylene in this tissue then the reaction is far more efficient in apples than in vitro. The data also indicate that the amount of linolenate does not change greatly during the period of time ethylene production was measured and also that a C 16 fatty acid with two double bonds is not present in the fatty acids extracted from the apple. If ethylene does arise from linolenic acid it does so from a small pool of activated (peroxidized) acid whose level is maintained by a rapid turnover of the activated fatty acid.

Naphthaleneacetic acid (NAA) is known to stimulate ethylene production from peas and beans.<sup>5</sup> It was of interest then to determine if increased rates of ethylene production would have any effect on linolenic acid levels from plants treated with NAA. Week-old etiolated pea and bean (cotyledons removed) plants were sprayed with  $2 \times 10^{-3}$  M NAA until runoff. Treated plants and controls were placed in containers for 20 hours, after which time ethylene evolution was measured and fatty acids were extracted. Representative chromatograms of the fatty acids of these plants are shown in Figures 3 and 4. No significant change was noted in the levels of any particular fatty acid between treated and control plants. Although care was taken to have the extraction of fatty acids quantitative, it was not possible to tell if changes in ethylene production between treated and controls were reflected in levels of linolenic acid (Table 2).

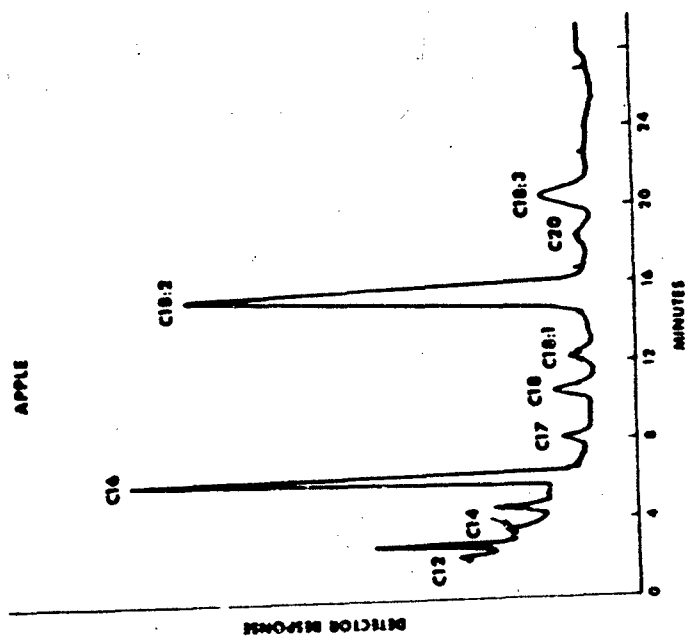


Figure 2. Fatty Acids from *Malus pumila* Mill.

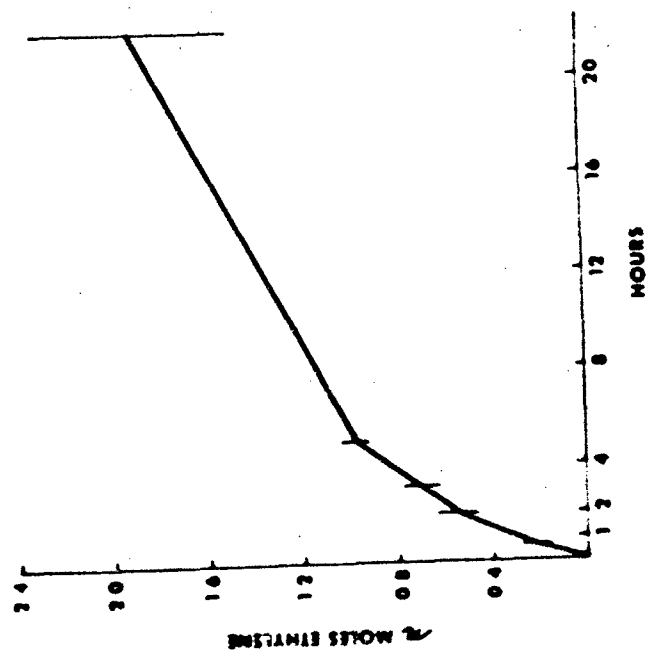


Figure 1. Ethylene Production from Peroxidized Linolenic Acid. 0.88  $\mu$ mole peroxidized (0.06 mole  $O_2$ /mole fatty acid) linolenic acid; 50  $\mu$ moles sodium ascorbate; 125  $\mu$ moles potassium phosphate buffer, pH 7.3; liquid volume, 2.5 ml; helium gas phase, 25 C. Vertical lines indicate extent of standard error.



TABLE 1. COMPARISON OF ETHYLENE PRODUCTION AND LINOLENIC ACID LEVELS FROM MALUS PUMILA MILL. VAR. EASTERN DELICIOUS

Experiment No.	Linolenic Acid, nmoles Fresh Weight, grams		Ethylene at 20 hrs, nmoles Fresh Weight, grams
	0 hours	24 hours	
1	50	33	8.8
2	32	48	15

TABLE 2. COMPARISON OF LINOLENIC ACID LEVELS AND ETHYLENE PRODUCTION FROM NAA-TREATED PLANTS

Experimental Material	Linolenic Acid, nmoles Fresh Weight, grams		Ethylene at 20 hrs, nmoles Fresh Weight, grams	
	Control	$2 \times 10^{-3}$ M NAA	Control	$2 \times 10^{-3}$ M NAA
<u>Pisum sativum</u> L.	340	130	0.05	7.1
<u>Pisum sativum</u> L.	140	260	0.05	2.2
<u>Phaseolus vulgaris</u> L.	570	480	0.18	14

The results with clupanodonic acid and other compounds containing terminal  $\text{CH}_3\text{-CH}_2\text{-CH=}$  groups indicated that the activity of linolenic acid is due only to this particular configuration. The possibility that ethylene may arise from this particular type of structure in nature remains open. Observing that a variety of other compounds such as ethylene oxide, diethyl ether, ethanol, and methionine have been reported<sup>1</sup> to produce ethylene suggests other alternatives. Any scheme that postulates linolenic acid as a precursor of ethylene in apples is unattractive because, first, the in vitro reaction is inefficient (0.02% after 20 hours) and the ratio of ethylene production to linolenic acid levels in apple is high (20% after 20 hours); and second, no C 16 fatty acid with two double bonds occurs in apple tissue. The fact that linolenic acid must be peroxidized<sup>1</sup> before it serves as a source of ethylene raises the question of whether fatty acid peroxides occur in normal tissues. Desai and Tappel<sup>6</sup> have shown that enzymes and subcellular particles are damaged by peroxidized fatty acids. However, there are numerous reports that peroxidized fats do occur in vivo.<sup>7</sup>

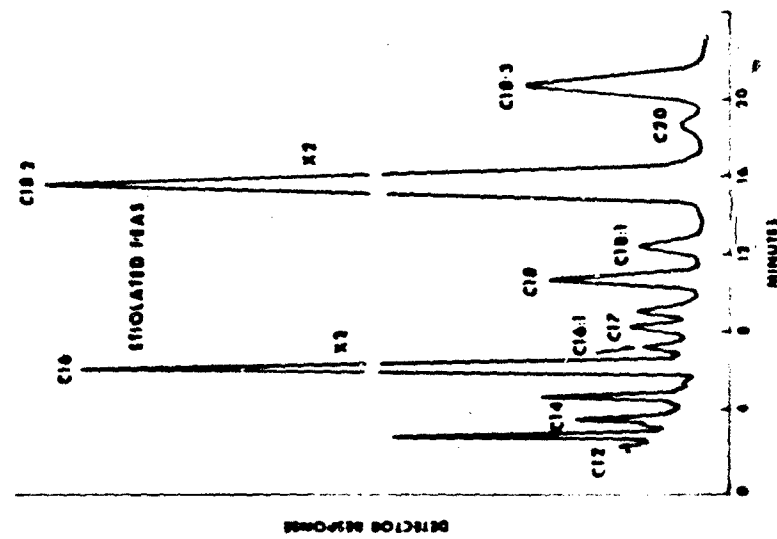


Figure 3. Fatty Acids from Etisolated Plasm sativum L.

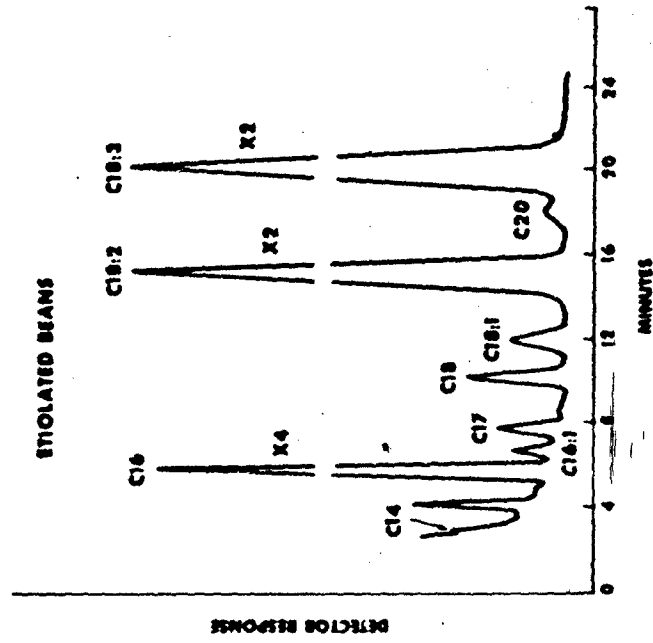


Figure 4. Fatty Acids from Etisolated Phaseolus vulgaris L.

In conclusion, the evidence indicates that linolenic acid does not serve as a precursor of ethylene. Final proof of this depends on measurements of rates of linolenic acid turnover and the extent to which peroxidized linolenic acid occurs in nature.

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